amplitude oscillation (frequency: 1 −100Hz) and determined elastic and viscous moduli. Results showed that hysteresis was greatly increased in the KO over the WT (for example at a speed of 100 %/s and an amplitude of 0.3 μm/sarcomere, hysteresis was 1549 ± 379pJ/mm^2/sarcomere vs. 401 ± 94pJ/mm^2/sarcomere; $p<0.05$). It can be calculated that this differences in hysteresis is analogous to an energy difference in a 24 hour period of 600 BPM of ~16cal, or ~30% of the total energy consumed by the heart. We conclude that the N2B element greatly reduces energy loss during stretch/shortening cycles of the beating heart.

2849-Pos

Single Molecule Analysis of PKC Phosphorylation of Titin's PEVK Domain

Brian Anderson[1], Julius Bogolomovas[2], Siegfried Labeit[2], Henk Granzier[1].
[1]University of Arizona, Tucson, AZ, USA, [2]Universitätsmedizin Mannheim, Mannheim, Germany.

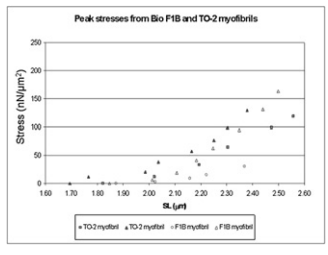
Titin's I-band region contains three spring-like domains that are primarily responsible for the development of passive tension in cardiac muscle. PKC phosphorylation targets one of these I-band domains_the PEVK region, which is rich in proline (P), glutamate (E), valine (V), and lysine (K). It has been shown that two serine residues within the PEVK are targeted by PKC phosphorylation, S26 and S170. We investigate the effects of PKC phosphorylation of these two residues on the single molecule level using force-extension curves generated by atomic force microscopy (AFM). We constructed four recombinant proteins: two PEVK single mutants (S26A and S170A), a PEVK double mutant (S26AS170A), and a wild-type PEVK segment. All constructs are flanked by immunoglobulin-like domains, Ig27 and Ig84, and the unfolding of these domains generates a single molecular ''fingerprint''. The force-extension curve leading up to the first unfolding peak describes the force-extension relationship of the PEVK. Preliminary data suggests that mutating either serine residue alters PEVK resistance to extension, which is quantified by the molecule's persistence length (PL). Wild-type PEVK underwent a large decrease in its PL after phosphorylation by PKC (by ~50%), and both single mutants have PLs similar to that of phosphorylated wild-type PEVK. Furthermore, phosphorylation of both single mutants resulted in a small PL decrease. Phosphorylation decreased PL for the S26A mutation by 16% (from 0.55 ± 0.02 to 0.46 ± 0.02 (mean ± SE)), and the serine-170 mutation PL by 11% (from 0.53 ± 0.04 to 0.47 ± 0.03). The double mutant was not affected by PKC (from 0.51 ± 0.04 to 0.51 ± 0.03). We conclude that both serines play a structural role in determining the relationship between longitudinal force and PEVK extension, and that this role is modulated through phosphorylation by PKC.

2850-Pos

Passive Stress in Myofibrils from Cardiomyopathic Hamsters

Audree McKenzie, Walter Herzog.
University of Calgary, Calgary, AB, Canada.

Dilated cardiomyopahty (DCM) is a frequent heart disease characterized by cardiac dilation and contractile dysfunction. The Bio TO-2 hamster is a genetic animal model of human DCM. The purpose of this research is to study the progression of DCM by comparing over time the passive mechanical properties of left ventricular wall myofibrils from TO-2 hamsters to those from F1B control hamsters. To date, we measured the passive stress-sarcomere length relations for two myofibrils each from experimental and control animals aged 36 weeks. Myofibrils were attached at one end to a glass needle controlled by a motor for stretching, and at the other end to a silicon-nitride nanolever of known stiffness for force measurements. Sarcomere lengths were measured from the myofibrillar striation patterns. Passive stresses in the experimental and control myofibrils were comparable. More passive mechanical experiments will be performed to confirm this result. In a single myofibril, titin is thought to be responsible for essentially all of the passive stress response to stretch (Linke et al., 1994; Bartoo et al., 1997). Titin depletion experiments and titin molecular weight determination will therefore also be performed to detect changes in titin isoform.



2851-Pos

Importance of Titin Based Viscosity in Cardiac Function: an Integrative Study on PEVK-Actin Interactions

Charles S. Chung[1], Methajit Methawasin[1], Joseph Popper[1], O. Lynne Nelson[2], Michael Gotthardt[3], Henk L. Granzier[1].
[1]Universityof Arizona, Tucson, AZ, USA, [2]Washington State University, Pullman, WA, USA, [3]Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.

Viscosity has recently been hypothesized as an important regulator of diastolic relaxation during isovolumic relaxation and early rapid filling. A viscous interaction between the proline-glutamic acid-valine-lysine (PEVK) rich region of titin and the actin filaments has been shown at the protein level, but the physiologic relevance of such an interaction is unclear. A novel PEVK kockout (KO) mouse was utilized in order to investigate PEVK-actin based viscosity. KO and wild-type (WT) skinned cardiomyocytes were isolated subjected to ramp-hold protocols. Our data showed that the viscosity measured via stress relaxation was more than 2x greater in the WT vs KO cells and that WT cells showed a 2x faster relaxation to the steady state force at each of 4 stretch speeds, a hallmark of viscosity. Also using KO and WT mice, we examined the presence of viscosity in the intact ventricle. Using both ramp-hold and sinusoidal oscillations, we found that, in intact hearts, WT displayed greater viscosity than KO hearts. Ramp-hold analysis on isolated hearts again showed a 2x faster relaxation in WT (36ms) vs KO (53ms). Sinusoidal analysis provides KO viscosity nearly 30% lower than WT (Viscous modulus WT=0.97 vs KO=0.65 [mmHg/uL]). Because physiologic stretch speeds were probed in stretches on cells and isolated hearts, we analyzed in-vivo echocardiographic measurements utilizing kinematic models of stiffness and viscosity known as the Parameterized Diastolic Filling Formalism. As expected with a truncated titin, stiffness increased in the KO mouse (WT=10,700 vs KO=12,400 mass normalized stiffness [1/s2]). Importantly, a 30% reduction in viscous properties (WT=143 vs KO=99 mass normalized viscosity [1/s]). Titin based viscosity driven by PEVK-actin interactions are present in the ventricle and could play an important role in diastolic function and dysfunction.

2852-Pos

Titin Isoforms and Titin-Based Stiffness in Diastolic Heart Failure

Judith Krysiak[1], Lars S. Maier[2], Burkert M. Pieske[3], Margaret M. Redfield[4], **Wolfgang A. Linke**[1].
[1]Ruhr University, Bochum, Germany, [2]University of Göttingen, Göttingen, Germany, [3]Medical University Graz, Graz, Austria, [4]Mayo Clinic, Rochester, MN, USA.

Diastolic heart failure (DHF) is a common heart disease characterized, e.g., by delayed relaxation, impaired left ventricular (LV) filling and increased LV stiffness. Titin is an established contributor to LV stiffness, but little is known about the protein's contribution to altered diastolic function in DHF. We investigated LV tissue samples of several animal models of DHF, as well as interventricular septum samples of aortic stenosis (AS) patients, for titin-isoform composition by loose-gel electrophoresis and titin-based stiffness by skinned-fiber mechanics. Induction of diastolic dysfunction in a small animal model, the ''two kidney one clip'' (2K1C) rat, which develops LV hypertrophy due to chronic afterload increase, caused no significant changes in the titin-isoform expression pattern, both 6 weeks and 8 months following surgery (~6% N2BA in both 2K1C and SHAM-operated LV, the remainder being N2B isoform; N2B contains a stiffer, N2BA a more compliant titin spring). Similarly, in a volume-overload mouse model created by aortocaval fistula surgery, cardiac titin isoforms remained unaltered compared to SHAM-operated animals (18.5% vs. 19.8% mean N2BA). However, in an old dog model (8-12 years) made hypertensive by bilateral renal wrapping, the cardiac N2BA proportion was significantly lower ($41.6 \pm 4.9\%$; mean ± SD) than in normal old dog LV ($46.2 \pm 4.2\%$; $p<0.020$). Mechanical measurements revealed passive-stiffness modulations consistent with the magnitude of titin-isoform switching. In contrast, in human AS samples, the titin isoform composition showed $42.0 \pm 4.0\%$ N2BA, significantly higher than in location-matched normal donor hearts ($37.5 \pm 5.0\%$; $p<0.025$). We conclude that diastolic dysfunction is associated with changes in cardiac titin isoform composition in a large animal model and in humans. The direction and the magnitude of the isoform shift appear to be determined by multiple factors not excluding, but clearly not restricted to, hemodynamic overload.

2853-Pos

Characterization of a Mutant Rat Model with Altered Titin Isoform Expression

Wei Guo, Jonathan M. Pleitner, Kurt W. Saupe, Timothy A. Hacker, Marion L. Greaser.
University of Wisconsin-Madison, Madison, WI, USA.

Titin isoform expression is related to human cardiac disease. A mutant rat model with dramatically altered titin isoform expression has been described (Greaser et al. J Mol. Cell. Cardiol. 44:483, 2009), and the ultrastructural and physiological properties of mutant and wild type rats were compared in this study. Electron micrographs of homozygous mutant ventricles showed normal structure in most areas, but occasional regions of Z line streaming, myofibrillar disarray, lipofuchin granules, and myofibril degeneration were observed as found previously in human heart failure. Dobutamine administration caused an increased heart rate in wild type (Wt), heterozygotes (Ht) and homozygote mutants (Hm), but